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Synaptotagmin IV

Biochemistry, Genetics, Behavior, and Possible Links to Human Psychiatric Disease

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Abstract

We isolated the rat synaptotagmin IV (Syt IV) cDNA in a screen for sequences that are specifically induced in neuronal cells. The Syts are a large family of genes thought to mediate synaptic function. Syt IV is brain-specific, induced in hippocampus by depolarization, and predominantly vesicular. To assess the function role of Syt IV in vivo, we generated Syt IV(–/–) mutant mice. Syt IV (–/–) mice are viable and appear normal, indicating this gene is not essential for survival or gross development. However, Syt IV (–/–) mutants, when compared to wild-type littermates, have deficits in fine motor coordination and hippocampus-dependent memory, suggesting Syt IV has a role in normal brain function. The human Syt IV ortholog maps to a region of chromosome 18 previously associated with the human psychiatric disorders, schizophrenia and bipolar disease. These results suggest that Syt IV is required in certain types of neurons for optimal functionality, that perturbations in the levels of Syt IV can result in memory loss in mice, and that Syt IV alterations may lead to psychiatric disease in humans.

Index Entries: Synaptotagmin IV; depolarization-inducible; synaptic plasticity; learning and memory; hippocampus; schizophrenia.

Introduction

Synaptic plasticity, the ability of neurons to modify synaptic output in response to prior electrical stimulation, is thought to be an important mechanism underlying memory formation *(1)*. Memory storage in brain may be encoded by changes in synaptic strength between neurons activated by learning. Neuronal activity, evoked in vivo by electrical stimulation or by learning, can promote long-lasting changes in

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synaptic function. For example, neurons of the hippocampus (HC) exhibit a long-lasting enhancement of synaptic efficacy, or long-term potentiation (LTP), following regular bursts of high-frequency stimulation *(2).* LTP is considered a cellular model for learning and memory *(3).* Similarly, synapses in the amygdala, a locus of fear response in the mammalian brain, are activated during conditioned-fear learning *(4).*

Long-term memory formation and the induction of long-lasting forms of LTP can be disrupted by inhibitors of transcription and translation *(5,6),* suggesting that *de novo* gene expression is required to establish and/or maintain the stable synaptic changes that underlie these processes. Immediate-early genes (IEGs) are genes whose transcription requires no intervening protein synthesis and utilizes pre-existing, latent signaling pathways and transcription factors *(7).* Activity-induced IEGs are likely to have an important role in the synaptic change associated with long-term memory and long-lasting LTP. We *(8),* and others *(9,10),* are interested in identifying and characterizing activity-inducible genes as potential mediators of synaptic plasticity. Indeed, many studies have identified so-called "candidate plasticity genes" *(11).* Analysis of candidate plasticity gene expression in models of LTP and behavioral learning has provided insight into the molecular mechanisms of neuronal plasticity.

This review describes a member of the synaptotagmin (Syt) gene family, Syt IV, which we isolated in a screen for inducible neuron-specific IEGs in the rat pheochromocytoma 12 (PC12) cell line. PC12 cells are a neuronal model system routinely used to study neuronal signal transduction *(12),* sympathetic neuron development *(13),* and evoked neuronal secretion *(14).* We describe the identification of the rat Syt IV cDNA as a depolarization-inducible gene; our use of a Syt IV-specific antiserum, to investigate biochemical properties of the Syt IV protein; and the creation of a Syt IV (–/–) knockout mouse, to assess the functionality of Syt IV in vivo. We have also recently identified a human Syt IV ortholog, and have mapped the human Syt IV gene to a chromosomal locus previously associated with schizophrenia (SZ) and bipolar disease. Because Syt IV is inducible by neuronal activity and appears to modulate synaptic function, the authors think Syt IV is an excellent candidate plasticity gene that may have a role in activity-induced neuronal plasticity, memory, and human psychiatric disease.

Syts Comprise a Gene Family

The Syts are a large family of proteins that function in vesicle trafficking. To date, 12 Syt isoforms have been identified *(15).* Syts are characterized by an intravesicular N-terminus, a short transmembrane domain, and a cytosolic region comprised of two structurally similar, but functionally distinct, regions homologous to the C2 domain originally described in protein kinase C *(16).* Many Syts are synaptic vesicle proteins *(17,18).* Through their C2 domains, Syts bind calcium (Ca) and phospholipid, and interact with a variety of presynaptic effector molecules *(19).*

Disruption of the murine Syt I gene *(20),* or the *Drosophila* and *Caenorhabditis elegans* Syt genes *(21,22),* results in neonatal lethality (mice) or severe paralysis (flies), because of a dramatic impairment in evoked, Ca-dependent synaptic release. In addition, the *Caenorhabditis elegans* Syt mutants exhibit reduced retrieval of synaptic vesicles from the plasma membrane, following depolarization *(23).* These observations suggest that Syts function both as a Ca regulator in the exocytotic fusion reaction and as part of the endocytotic vesicle retrieval apparatus.

Syt IV Is Inducible by Depolarization

The cDNA encoding rat Syt IV was isolated using differential screening and subtractive hybridization, in a screen for neuron-specific IEGs from PC12 cells *(24).* As part of an initial description of the Syt IV clone, we demon-

strated that Syt IV mRNA is rapidly and transiently inducible in PC12 cells by high potassium (K) depolarization and by forskolin, an activator of adenylyl cyclase *(24).* By comparison, Syt I mRNA levels are unchanged under these conditions *(24).* We also find that the human Syt IV gene is inducible in the human neuroblastoma, SK-N-SH, by K depolarization and by forskolin *(25).* However, Syt IV is not induced by nerve growth factor (NGF) or tetradecanoyl phorbol acetate (TPA), agents that induce differentiation of PC12 and SK-N-SH cells, respectively *(24,25).*

To determine if Syt IV is an IEG, we analyzed the induction of Syt IV mRNA in the presence of a protein synthesis inhibitor, cycloheximide. In both PC12 and SK-N-SH cells, high K depolarization and forskolin stimulation induce Syt IV mRNA, even in the presence of cycloheximide, indicating that Syt IV is an IEG in both rat and human cells *(24,25).*

We examined the depolarization inducibility of the Syt IV gene in vivo, using a kainic acid seizure model. Kainic acid is an agonist of the kainate-type glutamate receptor, which causes neuronal depolarization and seizures approx 30 min after its systemic administration. As detected by *in situ* hybridization, Syt IV mRNA levels reach maximal levels, in the HC and piriform cortex, 4 h after the onset of seizures *(24).* In contrast to adults, Syt IV is not well-induced by kainic acid in postnatal d 7 (PN7) animals, perhaps because the basal levels of expression are constitutively high *(26).* As was seen in PC12 cell culture, Syt I mRNA levels are unchanged in vivo, following stimulation in both adult and PN7 animals *(26).*

Syt IV Has Unusual, Conserved Substitution in C2A Domain

Syt IV has a stereotypical domain structure that includes a transmembrane region and a large cytosolic domain with tandem C2 repeats. Within the rat Syt family, Syt IV is most homologous to Syt XI (66% identity), least homologous to Syt III (22% identity), and 40% identical to Syt I *(15).* Rodent and human Syt IV orthologs are nearly 90% identical throughout the entire open reading frame *(25); Drosophila* Syt IV has lower levels of identity with orthologs within the C2A domain (Fig. 1).

The three-dimensional structure of the Syt I C2A domain revealed that the Ca-binding pocket is anchored by five aspartic acid residues *(27).* Syts containing all of the aspartic acid residues bind calcium (Ca), and interact with effector molecules in a Ca-dependent fashion; e.g., Syt I, through its C2A domain, binds the presynaptic plasma membrane protein syntaxin I in a Ca-dependent manner *(28).*

Syt IV and Syt XI have a Ser substitution at the third of five aspartic-acid residues in the Ca-binding pocket which renders these domains unable to bind Ca *(29).* This Ser substitution is found in the *Drosophila* and *C. elegans (30),* rat *(24),* mouse *(31),* and human *(25)* Syt IV orthologs; the substitution is conserved throughout evolution (Fig. 1). By mutating this Ser residue of Syt IV and Syt XI C2A to aspartic acid, a Ca- and phospholipid-binding activity can be introduced into these C2A domains *(29).* Thus, evolution has selected against Ca-dependent activity in the Syt IV and Syt XI C2A domains with a single amino acid change, while retaining the residues required for Ca-independent effector function. Indeed, the authors have demonstrated that the Syt IV C2A domain can interact with syntaxin I in a Ca-independent fashion *(32).* Moreover, microinjection of the Syt IV C2A domain into PC12 cells specifically blocks Ca-mediated secretory granule vesicle fusion with the presynaptic plasma membrane *(32).* Therefore, Syt IV, by virtue of the Ser substitution and its altered Ca-binding properties, may modulate the Ca sensitivity of Syt complexes and the probability of vesicle fusion in vivo.

The C2B domain of Syt IV, like many other Syts, interacts with numerous effectors, including other Syts *(32,33),* AP-2 *(18),* and N- and P/Q-type Ca^{2+}-channels *(33).* Syt IV homo-

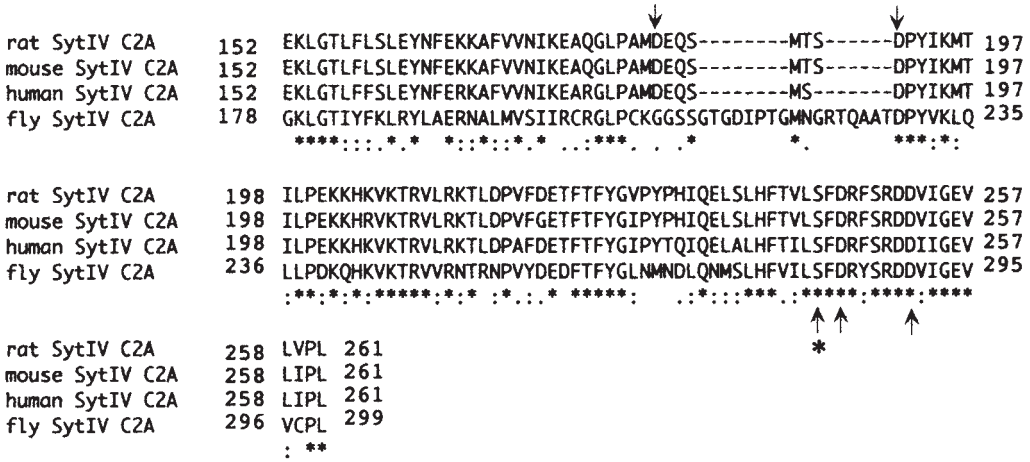

Fig. 1. Amino acid alignment of the rat *(24),* mouse *(31),* human *(25),* and fly *(30)* Syt IV C2A domains. The position of the conserved Ser residue is indicated with a large asterisk. The relative positions of the five aspartic acid residues in the Ca-binding motif within the C2A domain are indicated with arrows. Sequences were aligned with the CLUSTAL protein sequence alignment utility.

*= identical
:= strongly conserved
.= weakly conserved

oligomerization and hetero-oligomerization with Syt I is Ca-dependent *(32)*, indicating that the C2B domain of Syt IV is able to bind Ca. The Ca-independent interaction of Syt C2B domains with AP-2, a protein that participates in clathrin-mediated endocytosis, provides biochemical support for the hypothesis that Syts function in vesicle reuptake.

Syt IV Is Brain-specific

All Syts are expressed, to some extent, in brain *(19).* Numerous Syts have now been described outside the nervous system *(34).* We find, by Northern analysis, that Syt IV mRNA is expressed specifically in the rat neuroendocrine system, including brain and pituitary, and is not detectable in nonneuronal tissues (Fig. 2). Similar to rat Syt IV, human Syt IV mRNA is also restricted to brain, and is not found in nonneuronal tissues *(25).* Within brain, Syt IV is generally expressed in a rostral-to-caudal gradient, with the highest levels of expression observed in HC and lower, but significant, levels in cortex and cerebellum *(24).* Syt IV is expressed in CA3 pyramidal cell fields of the HC, in the granule and Purkinje cells of cerebellum, and in cortical layers II, III, and V *(35).*

Syt IV is the predominant Syt isoform in the HC and neocortex during the first week of post-natal development *(35).* The levels of Syt IV steadily decline into adulthood. In contrast, Syt I levels gradually increase and reach a stable maximum by PN15–20 *(35).* The spatial and temporal expression pattern of Syt IV throughout development led to the hypothesis that this gene may have a role linking electrical activity in neurons to synaptogenesis and axonal connectivity *(35).*

Syt IV Protein Is Predominantly Vesicular

To characterize the biochemical properties of the endogenous Syt IV protein, we developed a Syt IV antiserum. This antiserum specifically recognizes recombinant Syt IV in

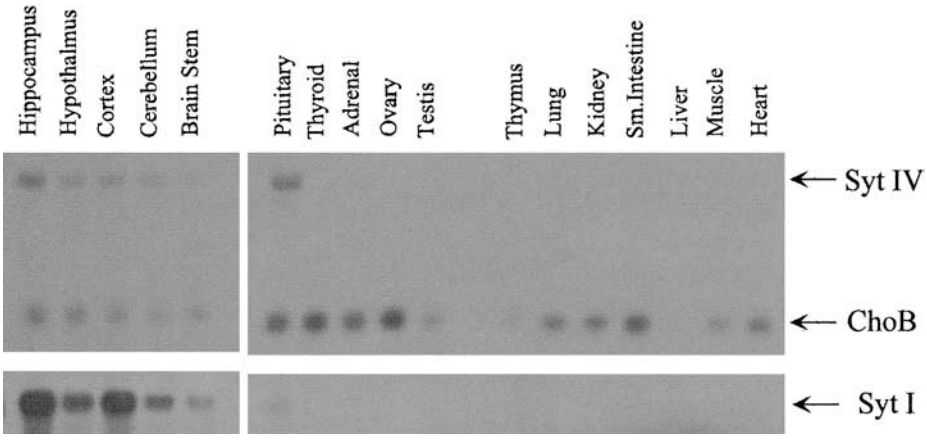

Fig. 2. Tissue distribution of Syt IV and Syt I mRNA. 10 µg total RNA from each of the indicated tissues or brain regions were subjected to Northern blot analysis, as described in ref. *(24).* The ChoB message serves as a loading control. Sm.Intestine, small intestine.

a large panel of other Syts *(32),* and interacts with a single 46-kDa band in stimulated PC12 cell extracts *(36).* Syt IV protein is not readily detectable in unstimulated PC12 cells. Concomitant with increases in mRNA, Syt IV protein levels increase rapidly and transiently in PC12 cells following K depolarization or forskolin stimulation *(36).* Taking advantage of the rapid incorporation of radioactive amino acids into newly synthesized Syt IV protein, we estimate that the rate of Syt IV protein synthesis increases 3–4-fold, following stimulation in PC12 cells *(36).* Radioactively labeled Syt IV is rapidly degraded in PC12 cells, with a metabolic half-life of approx 2 h, under these conditions *(36).* By comparison, other presynaptic proteins have half-lives of >8 h indicating that Syt IV is a relatively labile presynaptic protein. Ibata et al. *(37)* have confirmed the size and inducibility of Syt IV in PC12 cells, and have shown that the protein is also induced by depolarization in HC neurons.

Using anti-Syt IV antiserum, we investigated the subcellular localization of Syt IV protein in PC12 cells. We demonstrated that the Syt IV protein is detected in the neurites of NGF-differentiated PC12 cells following K depolarization (Fig. 3) or forskolin stimulation (unpublished observations), which is consistent with the notion that Syt IV is localized to secretory vesicles. Syt IV co-localizes with Syt I in the neurites of NGF-differentiated PC12 cells *(32).* Similarly, Syt IV distributes with known vesicle markers across sucrose density gradients and can be isolated in whole-vesicle immunoprecipitations with anti-Syt I antibodies *(36).* Thus, our data, and that of others *(30,38),* indicate that Syt IV is a vesicular protein.

Recently, Ibata et al. *(37)* and Berton et al. *(39)* reported that the localization of Syt IV in unstimulated neurons was primarily perinuclear, i.e., in endoplasmic reticulum (ER) Golgi complexes. In both studies, axonal and synaptic staining of Syt IV was also observed, although Syt IV was poorly co-localized with Syt I. These authors suggest that Syt IV, much like Syt III and Syt VI *(40),* is excluded from synaptic vesicles. In our fractionation and immunofluorescence experiments, Syt IV is also detectable in ERGolgi membranes (Fig. 3). This Syt IV fraction is attributed to newly synthesized protein that has not been transported to distal organelles. Indeed, when compared to *continuous* forskolin stimulation (unpublished observations), Syt IV staining in the ER–Golgi is substantially reduced, by

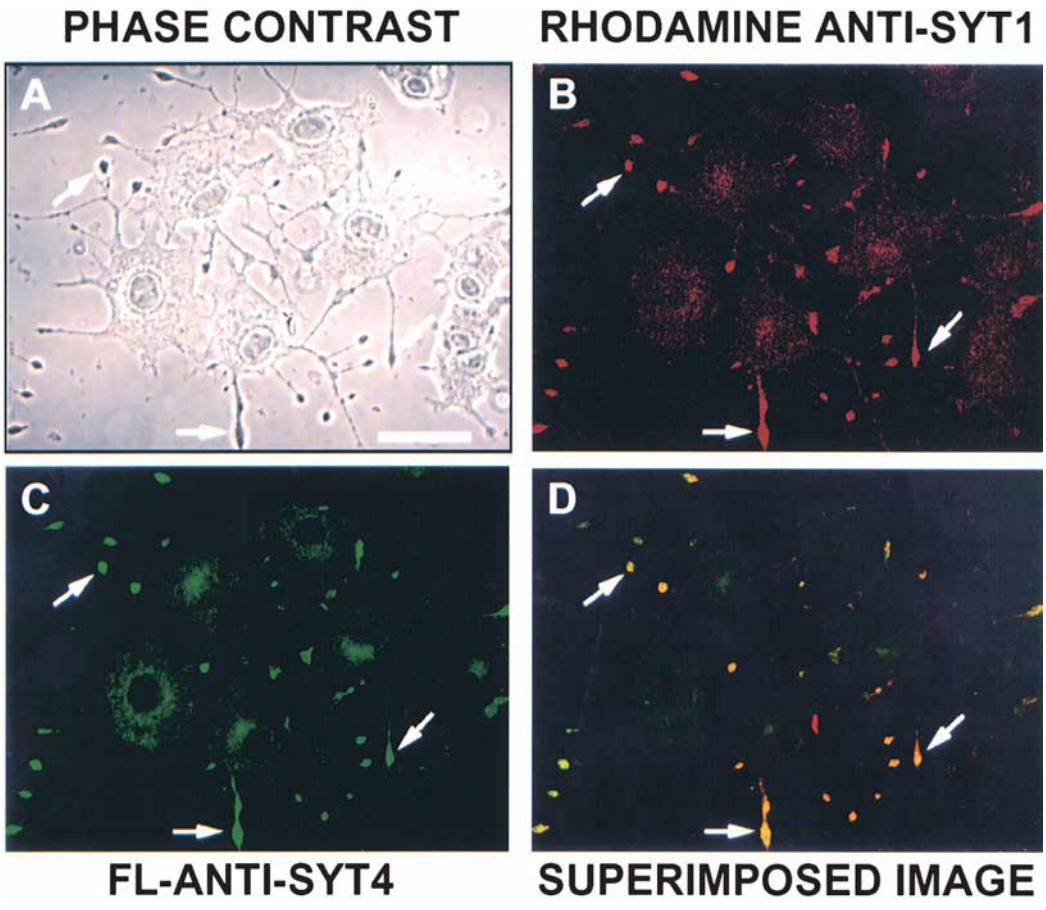

Fig. 3. Subcellular localization of Syt IV and Syt I protein in PC12 cells. 2 h after depolarization with KC1, cells were processed for immunofluorescence, as described in ref. *32*. **(A)** Phase-contrast image of a representative NGF-differentiated PC12 cell. Cells were stained for endogenous Syt I **(B)** and, using our specific antiserum, for induced Syt IV **(C).** Images from **(B)** and **(C)** were merged in **(D),** to show areas of co-localization between Syt IV and Syt I in the tips of neurites, which are indicated with arrows. Note also the presence of the perinuclear ER–Golgi Syt IV staining. (Reproduced with permission from ref. *32*.)

following a pulse of depolarization with a 2-h "chase" period. Without adequate synaptic stimulation, i.e., depolarization, to promote vesicle transport and recycling, it is possible that the high levels of Syt IV found in neonatal neurons are inefficiently transported to distal organelles. Much like our result in PC12 cells, Ibata et al. *(37)* reported increased levels of Syt IV protein in HC neurites following depolarization and a 90-min recovery period. Nevertheless, it is possible that portion of steady-state Syt IV protein is not associated with synaptic vesicles.

Syt IV Can Modulate Synaptic Function

Perhaps the most insightful experiments addressing the biochemical and functional properties of Syt IV have come from Littleton et al. and their work in *Drosophila (30).* Fly Syt IV,

like its mammalian counterpart, has the Ser-for-Asp substitution in the C2A domain, and does not bind Ca and phospholipid. Moreover, Syt IV hetero-ologomerizes with Syt I, and inhibits the ability of Syt I to interact with Ca and phospholipid *(30).* When overexpressed at the fly neuromuscular junction, Syt IV, but not Syt I, caused a significant reduction in evoked secretion. Overexpression of both Syt IV and Syt I decreased the number of spontaneous vesicle fusion events *(30).* These studies suggest that Syt IV can inhibit normal Syt function, in effect functioning as a dominant negative. Thus, the relative levels of Syt IV, upregulated by neuronal activity *(24),* may serve to attenuate synaptic responses. Syt IV may have evolved as an adaptive mechanism whereby neurons can attenuate synaptic output in response to overstimulation, i.e., seizures.

Syt IV Is not Essential for Survival

To analyze the role of Syt IV in vivo, we generated, using standard embryonic stem cell technology, mice harboring a homozygous disruption of the Syt IV gene *(41).* The breeding of mice heterozygous for the Syt IV mutation yielded near-Mendelian ratios of offspring: $^{+/+}$, 23%; $^{+/-}$, 56%; $^{-/-}$, 21% *(41).* Syt IV (–/–) mutant mice survive to at least 18 mo of age (unpublished observations), indicating that the Syt IV gene is not essential for long-term survival. Because Syt IV (–/–) mutant mice are viable, we were able to examine them in a variety of behavioral tests. Syt IV (–/–) mutant mice are indistinguishable from wild-type littermates by appearance and home cage behavior *(41).* In addition, Syt IV (–/–) mutant mice display no evidence of ataxia and are normal in a variety of tests of strength and balance (Fig. 4). Consistent with these behavioral observations, histological analysis of the brains of Syt IV (–/–) mutant mice reveals no gross structural abnormalities *(41).* More detailed structural analysis of brains from the Syt IV (–/–) mutant mice may reveal minor differences in brain anatomy.

Syt IV (–/–) mutant mice do have deficiencies in fine motor coordination *(41).* In the accelerating rotorod, a paradigm in which mice must remain upright on a rod whose rotational velocity increases over time, Syt IV (–/–) mutant mice exhibit an immediate and significant performance deficit which is maintained over five trials *(41).* The Syt IV (–/–) mutant mice improve their performance much like wild-type mice *(41),* suggesting motor learning for this task is intact. The Syt IV (–/–) mutant mice perform normally in a constant, low-speed rotorod test. These observations are consistent with the constitutive expression of Syt IV in cerebellum *(35)* and suggest the impaired rotorod performance derives from a deficit in motor coordination, rather than motor learning.

Syt IV Is Required in HC-dependent Memory

Previously, we postulated that Syt IV may modulate synaptic function and, therefore, learning and memory *(24,36).* Thus, the Syt IV (–/–) mutant mice were examined in learning and memory paradigms. First, we tested the Syt IV (–/–) mutant mice in a Pavlovian fear-conditioning model *(42–44).* In this paradigm, mice learn to fear an otherwise innocuous stimulus (the conditional stimulus), usually a context or a tone, by pairing it with an aversive stimulus, such as a foot shock (the unconditional stimulus). Following re-exposure to the conditional stimulus, trained mice exhibit a stereotypical fear response: the cessation of movement *(45).* Contextual conditioning is thought to be more sensitive to HC lesions *(46,47);* tone (cued) conditioning is more sensitive to amygdala lesions *(47).* When tested for contextual memory 24 h after training, Syt IV (–/–) mutant mice exhibit a statistically significant deficit in freezing compared to wild-type mice (Fig. 5A), indicating the mutant mice failed to remember the conditional stimulus–unconditional stimulus pairing *(41).* Because the Syt IV (–/–) mutant mice have

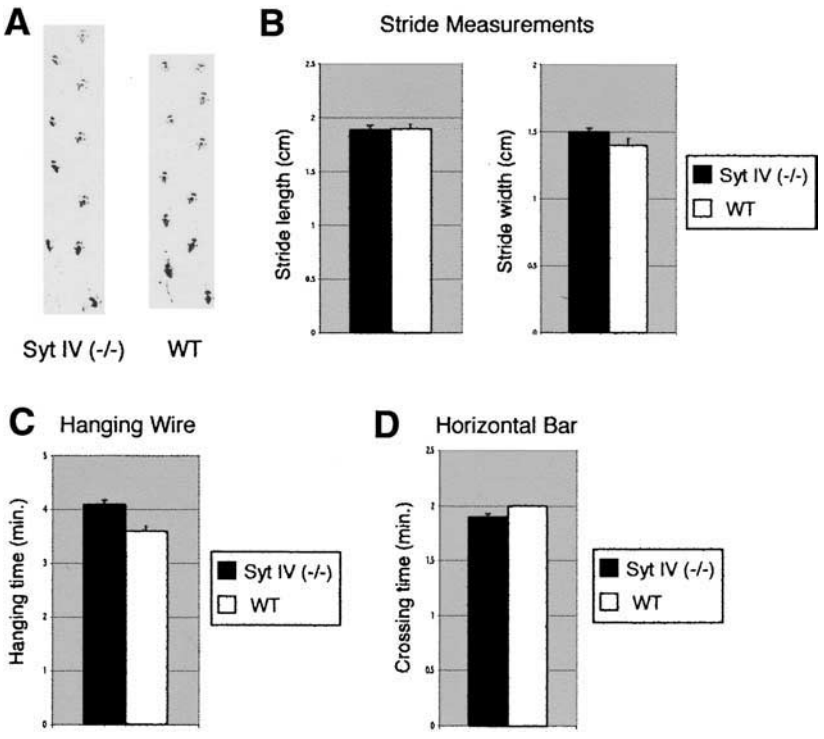

Fig. 4. Behavioral assessment of Syt IV (–/–) mutant mice. Syt IV (–/–) mutant mice show no evidence of ataxia, as seen in hind paw ink blot **(A),** or any significant difference in stride length or width **(B).** Syt IV (–/–) mutant mice have normal strength in the hanging wire test **(C)** and balance in **(D).** In **(B),(C),** and **(D),** data are expressed as mean ± SEM.

normal sensitivity to footshock, exhibit normal baseline freezing and activity, and can freeze to wild-type levels after overtraining *(41),* we conclude that their performance deficit in contextual conditioning has a mnemonic basis.

We explored the HC function of the Syt IV (–/–) mutant mice further, in another HC-dependent memory test: the social-transmission-of-food-preference paradigm *(48).* This task, which does not use aversive stimuli, exploits the natural tendency of mice to prefer foods that they have recently smelled on the breath of other mice, and tests the ability of mice to learn and remember this information. Syt IV (–/–) mutant mice show a decreased socially transmitted food preference, compared to wild-type mice, when tested 24 h after training *(41).* When tested immediately after training, however, the Syt IV (–/–) mutant mice display normal food preference, indicating the Syt IV (–/–) mutant mice can perform all aspects of the test normally, and suggesting the 24-h deficit was, again, a memory failure.

We also examined the Syt IV (–/–) mutant mice in other memory tests, including cued-fear conditioning (Fig. 5B; *47*) and conditioned-taste aversion *(49),* both of which depend on amygdala function. In both instances, Syt IV (–/–) mutant mice perform normally *(41),* suggesting amygdala function is not compromised in the Syt IV (–/–) mutant mice. From these memory studies, we conclude that the Syt IV mutation specifically perturbs the function of the neurons of the HC, a region of high Syt IV expression, and that this perturbation manifests in memory tasks known to require HC function.

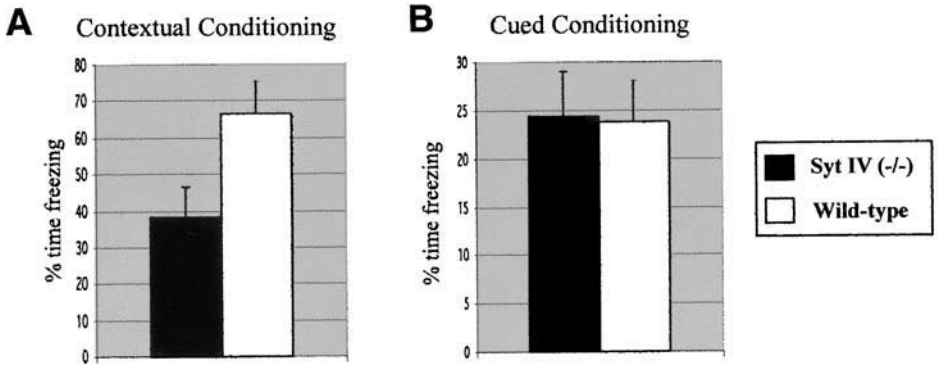

Fig. 5. Context and cued-fear conditioning in Syt IV (–/–) mutant mice. **(A)** 24-h after context training, in which mice were given a single 2-s footshock of 0.7 mA, Syt IV (–/–), mutant and wild-type mice were tested for context freezing, as described in ref. *(41).* Data shown are mean percentages of freezing during the entire 4-min context test. Data point and bars are mean ± SEM, respectively. **(B)** 24-h cued-conditioning in a separate group of Syt IV (–/–) mutant and wild-type mice. A single footshock as above, was co-terminated with a 10-s tone. Mice were tested in a novel context, as described in ref. *41.* Data shown are mean percentages of freezing during the entire 5-min test. Data points and bars are means ± SEM, respectively.

Human Syt IV May Be Linked to Psychiatric Disease

Because Syt IV (–/–) mutant mice have deficits in motor performance and HC-dependent memory, we sought to determine whether the human Syt IV is associated with any known neurologic diseases. Initially, a human sequence was identified and characterized which, based on homology and the presence of the serine residue at the appropriate position, we now consider a human ortholog of Syt IV *(25).* Using fluorescence *in situ* hybridization, the authors mapped the human Syt IV gene to human chromosome 18q12.3 *(25).* This region and nearby regions of chromosome 18 have been identified, through population genetics *(50),* small family studies *(51),* and epidemiology *(52),* as SZ and bipolar disorder susceptibility loci. How might Syt IV be related to the development of SZ? Patients with SZ often display memory deficits that have been attributed to HC dysfunction *(53).* Moreover, presynaptic abnormalities in neurotransmission are thought to be a basis for psychiatric disease *(54,55).* For example, altered dopamine neurotransmission has been observed in patients with SZ *(56).* We find that human Syt IV is expressed in dopaminergic brain regions *(25),* and rat Syt IV is found on the dopamine-containing vesicles of PC12 cells *(36),* suggesting Syt IV may regulate dopamine release in the human brain. In addition, decreased levels of mRNA-encoding presynaptic molecules *(57),* such as Syt IV *(58),* has been observed in the brains of patients with SZ, when compared to age-matched controls. Thus, abnormal levels of Syt IV may alter the presynaptic function neurons, leading to neurotransmitter dysregulation and the development of neurologic disease.

Future Directions

To further understand the role of Syt IV in mammalian neurotransmission, it will be important to characterize the electrophysiological properties of neurons from Syt IV (–/–) mutant mice. Our behavioral studies indicate that neurons of the HC and cerebellum are most affected by the loss of Syt IV; therefore, the basal physiologic and LTP-like properties

of neurons in the HC and cerebellum should be examined. Presynaptic and transcriptionally dependent forms of LTP, including HC mossy fiber long-lasting LTP *(59)* and cerebellar parallel fiber–Purkinje cell LTP *(60),* may prove most informative.

By using paradigms such as LTP or contextual learning and other pharmacologic stimuli, to induce Syt IV, it may be possible to establish the broader and perhaps more physiologically relevant parameters of Syt IV gene induction. The second messengers, Ca and Cyclic adenosine monophosphate (cAMP), lead to the induction of Syt IV, and may do so by activating the calcium/calmodulin-dependent kinase, protein kinase A (PKA), and the Ras mitogen-activated protein kinase pathways. It will be informative to examine the induction of Syt IV in the presence of specific pharmacologic inhibitors of these pathways. In this regard, PKA inhibitors have been reported to block the induction of Syt IV protein following depolarization of PC12 cells or HC neurons *(37).* Similarly, identifying the *cis*-acting elements within the Syt IV promoter region, and the transcription factors that bind these elements, will be helpful in defining the Syt IV transcriptional response. The authors have identified a near-consensus cAMP response element (CRE) in the Syt IV promoter (unpublished data). The CRE element is strongly responsive to high K depolarization and to forskolin *(12).* However, NGF, which only weakly induces Syt IV *(24),* also activates CRE elements *(61),* and leads to CRE-binding protein phosphorylation *(62).* Syt IV may also be regulated by other Ca-responsive elements that are not neurotrophin-responsive. For example, serum response elements, which do not contain a ternary complex factor family member binding site, can function as a Ca-responsive, but not NGF-responsive, element in neurons *(63).*

The Syt IV overexpression studies in *Drosophila* were informative enough to justify similar experiments in mammals. Advances in mammalian transgenic technologies now permit spatial and temporal control of transgene expression. For example, using the *Escherichia coli* tetracycline-controlled transactivator protein system *(64),* Mansuy et al. *(65)* developed a transgenic mouse strain in which the protein tyrosine phosphatase, calcineurin (PP-2B), is both HC-specific and temporally regulatable. By placing Syt IV into a similar transgenic strain, and promoting the rapid and reversible overexpression of the Syt IV protein, the consequences of acute Syt IV overexpression may be further understood, both in neuronal physiology and in mouse behavior. Because of the rapid induction kinetics of this system and the short metabolic half-life of Syt IV *(36),* it may be possible to monitor the LTP response and behavioral memory of, respectively, neurons and transgenic mice before, during, and after Syt IV overexpression.

Analyzing, in more detail, the relationship between Syt IV and SZ will be important. The development of experimental models for SZ has been difficult. Chronic administration of cocaine has been used in models of SZ and can promote long-lasting structural changes in the brain. Syt IV, which can be induced in the striatum by cocaine *(66),* has been proposed to participate in these structural changes *(67).* Thus, the Syt IV (–/–) mutant mouse may be a suitable mouse model for study of SZ. At the very least, Syt IV (–/–) mutant mice will provide neurons in which to assess the role of Syt IV in dopaminergic neurotransmission. A direct quantification of the levels of Syt IV in human SZ tissue will be problematic, because of the difficulty in obtaining human samples.

Acknowledgments

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